# Molecular Ecology Laboratory

Southwest Fisheries Science Center
8604 La Jolla Shores Drive
La Jolla CA 92037
Fax: 858-546-7003

---

## Laboratory Protocol

Protocol number: 3
Protocol description: DNeasy/Fastprep Extractions
Original reference: Qiagen and Qbiogene tissue extraction protocols
Original entry: Carrie LeDuc
Last updated: 23 October 23, 2003
Updated by: Carrie LeDuc

**Required materials:**
Fastprep Lysing Matris A tubes

Weigh boats

Razor Blades

Squirt Bottle of $mQH_2O$

Forceps

DNeasy tissue kit reagents

100% EtOH

**Required equipment:**
Fastprep homogenizer

Water bath at $55^0$ C

Heat block at $70^0$ C

Timer

Centrifuge

Vortex

**Procedure:**

1. Turn on the water bath to $55^0$ C and heat block to $70^0$ C.

2. Remove tissue from vial with forceps and wash by squirting with mQ water. Cut tissue on a clean weigh boat with a new razor blade. Chop the tissue into smaller pieces with the razor blade and use the tweezers to put the tissue into the orange capped Fastprep tube.
3. To a Fastprep tube add:
    a. Tissue
    b. 180ul Buffer ATL
4. Fastprep tubes a 5.0 speed for 45 seconds 2-3 times. Allow tubes to cool or put on ice before starting the Fastprep machine again. Repeat until tissue is pulverized.
5. Centrifuge tubes at low speed to remove bubbles.
6. Add 20ul PK (from DNeasy extraction kit) to the Fastprep tube.
7. Vortex.
8. Incubate tubes at $55^0$ C for 1 hr (or $37^0$ C overnight) in the water bath. Vortex occasionally.
9. Add 200ul Buffer AL to the Fastprep tube and vortex.
10. Incubate tubes at $70^0$ C for 10min on the heat block.
11. Add 200ul 100% EtOH to the Fastprep tubes and vortex.
12. Quick spin the Fastprep tube and pipet supernatant from the Fastprep tube into a DNeasy column.
13. Centrifuge at 8000rpm for 1min.
14. Discard collection tube containing flow-through and place column in a new collection tube.
15. Add 500ul Buffer AW1 to the column.
16. Centrifuge at 8000rpm for 1min.
17. Discard collection tube containing flow-through and place column in a new collection tube.
18. Add 500ul Buffer AW2 to the column.
19. Centrifuge at 13000rpm for 3min.
20. Discard collection tube containing flow-through and place column in a 1.7ml tube.
21. Add 100ul Buffer AE to the column and allow to sit for 1min.
22. Centrifuge at 8000rpm for 1min to elute DNA.
23. Repeat steps 21-22 with the same 100ul of Buffer AE.